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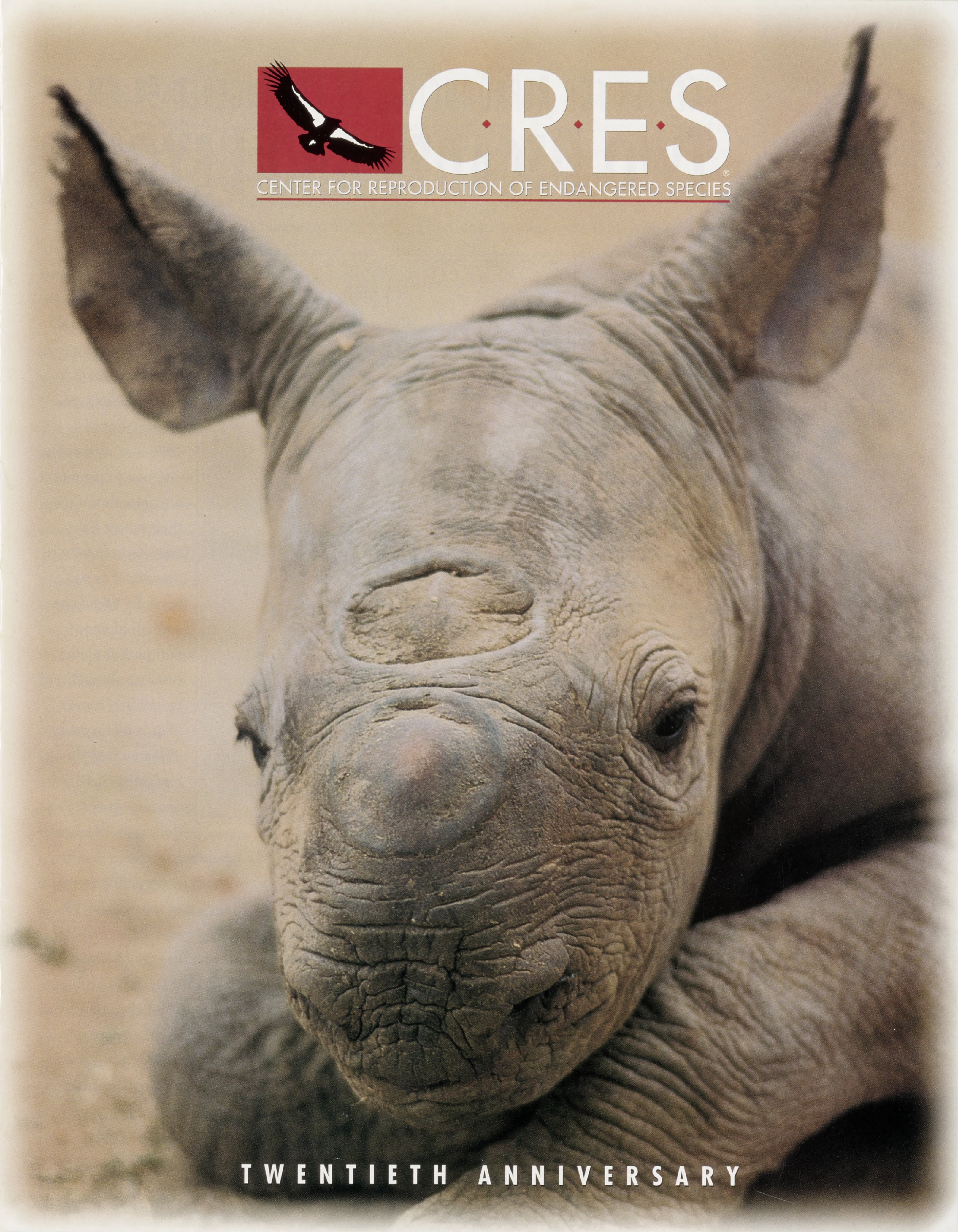
CENTER FOR REPRODUCTION OF ENDANGERED SPECIES



TWENTIETH ANNIVERSARY

1975–1995
20th
ANNIVERSARY
CRES

CENTER FOR REPRODUCTION OF ENDANGERED SPECIES
TWO DECADES AND BEYOND

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Cover: A young East African black rhinoceros *Diceros bicornis michaeli* photographed at the San Diego Wild Animal Park. Unlike white rhinos, black rhinos have not bred well in captivity. Ongoing rhino projects involve many CRES divisions, including behavior, endocrinology, virology, reproductive physiology, and genetics.

What makes the San Diego Zoo one of the world's great zoos? Most people would say it is the size and scope of the animal and plant collections. But, in addition to the San Diego Zoo and the San Diego Wild Animal Park, there is a third arm of the Zoological Society of San Diego, smaller and less well known to the general public. It is CRES—the Center for Reproduction of Endangered Species. CRES is the research department of the Society. Since its founding in 1975, CRES has been a magnet for top zoological scientists. The basic and applied research done here is a major reason for the acknowledged excellence of the San Diego Zoo. "CRES adds an important dimension to both the Zoo and the Park" said Dr. Werner Heuschele, CRES director. "It's what I hope all zoos will do eventually. They need to study the basic problem of the management of species for survival, as well as solving practical problems within the institution. Our success contributes to better management."

Because of international agreements and the United States Endangered Species Act of 1973—as well as the urgency of protecting animals within their native habitats—zoos must now learn to successfully breed the animals already within their collections or obtain them from other zoos. This is one reason why much of the research at CRES focuses on enabling endangered animals to reproduce. "We need to establish self-sufficient populations in captivity as well as in the wild, using all the knowledge we can gain to improve their future," Dr. Heuschele said. "We know very little about the biology of almost all nondomestic species," added Dr. John Phillips, deputy director of CRES. "The behavior, physiology, disease resistance, and genetics of endangered species are all high priority for us, not only because we know less about these species relative to others but also because there are so few of them."

HOW IT ALL BEGAN

Biological research has been one of the San Diego Zoo's missions ever since its founding in 1916. This was a novel approach, since zoos at that time were viewed largely as a place to exhibit exotic animals for the amusement and amazement of visitors. But the San Diego Zoo's principal founder, Dr. Harry Wegeforth, was a physician and a firm believer in science.

Even before the San Diego Zoo opened to visitors in 1922, Dr. Wegeforth approached well-known philanthropist Ellen Browning Scripps about funding. He was persuasive, and she donated $50,000 to erect a research facility, which was called the Zoological Hospital and Biological Research Institute. A commission was given to noted architect Louis Gill, whose two-story, award-winning building was dedicated in 1927. Although the Zoo constructed a new hospital in 1977, the research department—now named CRES—is still headquartered in the handsome, but cramped, 70-year-old Scripps Building, located behind the Old Globe Theatre in Balboa Park.

At first the Zoo's scientific work, as well as its veterinary care, was provided by local veterinarians and physicians. In 1929, the Zoological Society of San Diego became one of the first zoos to have a staff veterinarian—a notable step, even though he doubled as the San Diego County poultry pathologist. The Zoological Society established a research committee as early as 1925. The committee achieved greater prominence in 1932 when it was taken over by Rawson Pickard, M.D., who would chair it for the next 20 years. Most of the research committee's work consisted of approving requests from outside scientists for tissue or specimens. A veterinary pathologist, Dr. Lynn Griner, was added to the staff in 1964, making it possible for the first time to systematically study the health of the collection.

The Center for Reproduction of Endangered Species (CRES) owes its beginnings to the far-sighted planning and unflagging energy of the late Dr. Charles Schroeder, the Zoo's director from 1954 to 1972; to Dr. Kurt Benirschke, a physician who conducted pioneering work in human twinning and was Professor of Pathology and Reproductive Medicine at the University of California, San Diego; to Charles Bieler, who became Zoo director in 1973; and to the Society's board of trustees, who approved the vision for CRES.

In the early 1970s, Kurt Benirschke, M.D., became chairman of the research committee. A physician and pathologist by training, Dr. Benirschke and his committee, most of whom were local physicians or scientists, decided to challenge the board of trustees to "get serious about doing research here." The committee drew up a pair of "white papers" proposing a full-fledged research department.

Accepting the proposal, the trustees in effect told Dr. Benirschke, "It's a good idea—you do it." By then he was chairman of the UCSD pathology department, but Dr. Benirschke agreed to become director of the Zoo's research department.

His initial staff consisted of two technicians and a secretary, and he quickly recruited several scientists to join the fledgling department. With the help of students and volunteers, laboratory research work began just a week after moving in.

◀ Dr. Harry Wegeforth, the San Diego Zoo's principal founder, was a firm believer in science and succeeded in having an animal hospital building constructed on Zoo grounds. It opened in 1927.

> *"If everything was great in the wild, and animals were living and reproducing, there would be no need for us."*
>
> *—John A. Phillips, Ph.D., Deputy Director of CRES*

CRES TODAY

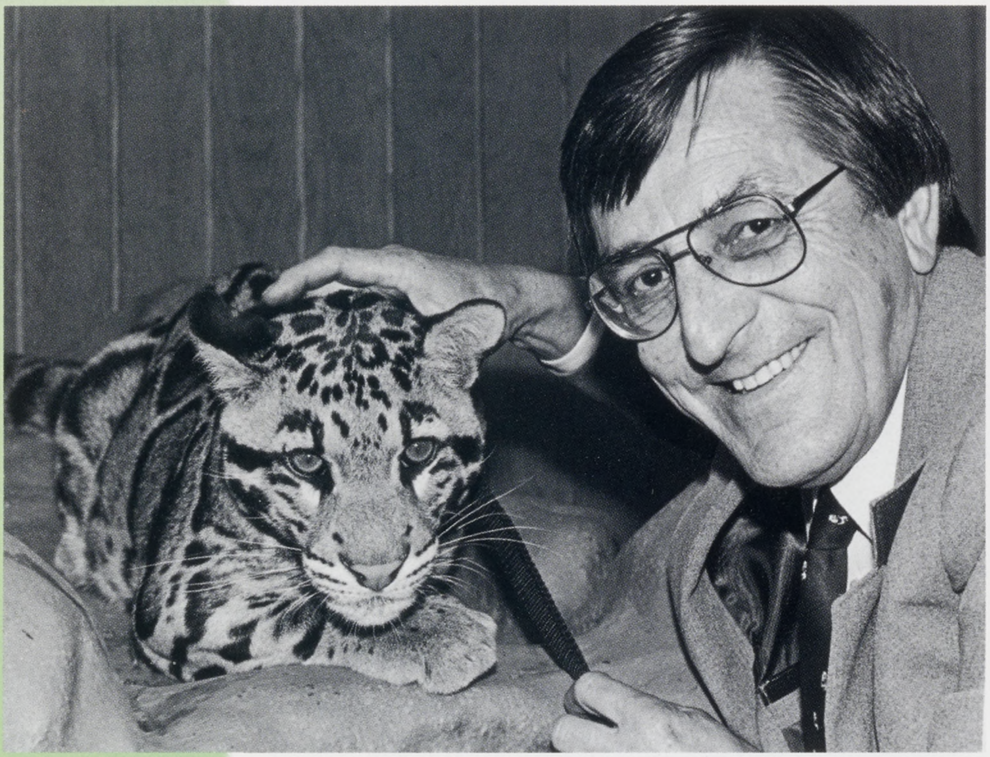
CRES has come a long way since that first week. About 34 people now work full-time at CRES, including a dozen senior scientists. They are supported by a small army of highly specialized laboratory associates and technicians, many of whom carry out significant research and publish scientific articles in their own right.

Besides the regular staff, there is a steady flow of new blood. Temporary positions at CRES may include:

Postdoctoral fellows—Scientists who recently received their Ph.D. or D.V.M. degree may come to CRES to carry out a specific study. These highly coveted assignments are usually for one or two years and are supported by an outside grant from a foundation, government entity, or private donor. Many "postdocs" come from other countries, and all add to CRES's valuable network of worldwide contacts when they go on to pursue independent work. In some cases, their association with CRES may become a long-term career. Several CRES division heads originally came here as postdoctoral fellows.

Students—Some CRES divisions have study or research programs for students at the high school, college, or graduate level.

Volunteers—Because of the exacting nature of the work done at CRES, most volunteers must be turned away. However, more than 50 skilled volunteers play an important role in the research of many divisions at CRES. "So much of the effort in studying behavior is in the long hours of sitting in front of a group of animals, waiting for behavior to accumulate," explained behaviorist Dr. Donald Lindburg. "We have a tremendous pool of talented, very dedicated volunteers. Some of our volunteers achieve a very high level of training."



▲ Dr. Werner Heuschele became director of CRES in 1986. He has had a long and distinguished career with the Zoological Society of San Diego as a veterinarian and head of the research department's virology laboratory. On the subject of the Earth's endangered creatures, Dr. Heuschele believes that "Our survival may well be linked with theirs."

CRES TOMORROW

What is ahead for CRES? Dr. Heuschele hopes to see the institution gain more recognition in its own right. Several departments need to be expanded, including microbiology and pathology, Dr. Heuschele said. More field studies, more postdoctoral fellows, and more students are needed, but working space is very limited.

A major priority is to completely fund the CRES endowment. When fully funded, it should provide most of the institution's operating money, which must now come out of the general budget of the Zoological Society of San Diego. "People must provide the means for the future survival of Earth's endangered creatures, since it is people who have been largely responsible for their decline, " Dr. Heuschele said. "Our survival may well be linked with theirs."

RESEARCH ON RARITIES

It is hard to do controlled, scientific studies with endangered animals. There are usually not enough of them to do a scientifically valid study, and besides, no one wants to take the slightest chance with a rare animal.

For example, when the Zoological Society received six young Komodo dragons to raise, it seemed a golden opportunity to evaluate the best diet for captive Komodos. In zoos, the large, carnivorous lizards are usually fed a diet of mice and rats, but in the wild the juveniles eat mostly insects and lizards. Might the ingested hair from the mice be causing health problems for the giant creatures? To test the theory, Zoo nutritionist Mark Edwards is working with CRES comparative physiologists to develop a turkey-based diet. Three of the young Komodos are being raised on the experimental diet and three on the traditional diet of mice. Comparing the growth and health of the two groups could indicate which is the better diet, thus improving the well-being of Komodo dragons here and at other zoos.

A frequent problem is how to encourage a pair of animals to mate. Often, it is best to leave the male and female together continually. But with some species, it is better to house them separately, except for the brief period when they actually mate. Choosing which method to use for a particular species can almost amount to informed guesswork. But a more scientific approach was tried with the Zoo's colony of pygmy lorises. Some pairs were housed together for a year, while other pairs were kept apart, except when the female was in estrus. The following year the arrangements were reversed for individual pairs. By tallying the number of pregnancies achieved, it should be possible to tell which method works better for this species.

Many CRES divisions mix studies on captive animals and laboratory work with field research. From pandas and gorillas to lizards and songbirds, CRES researchers and affiliated students have worked in many far-flung places. "We have a large presence in Latin America and a large presence in Africa," Dr. Phillips stated. "We are starting to do a lot of work in Australia. We don't have much room for additional personnel in our building here, but we are getting more involved in cooperative and foreign projects. Every year there are 30 or so projects proposed, and we can actually find funding for about 10 of them."

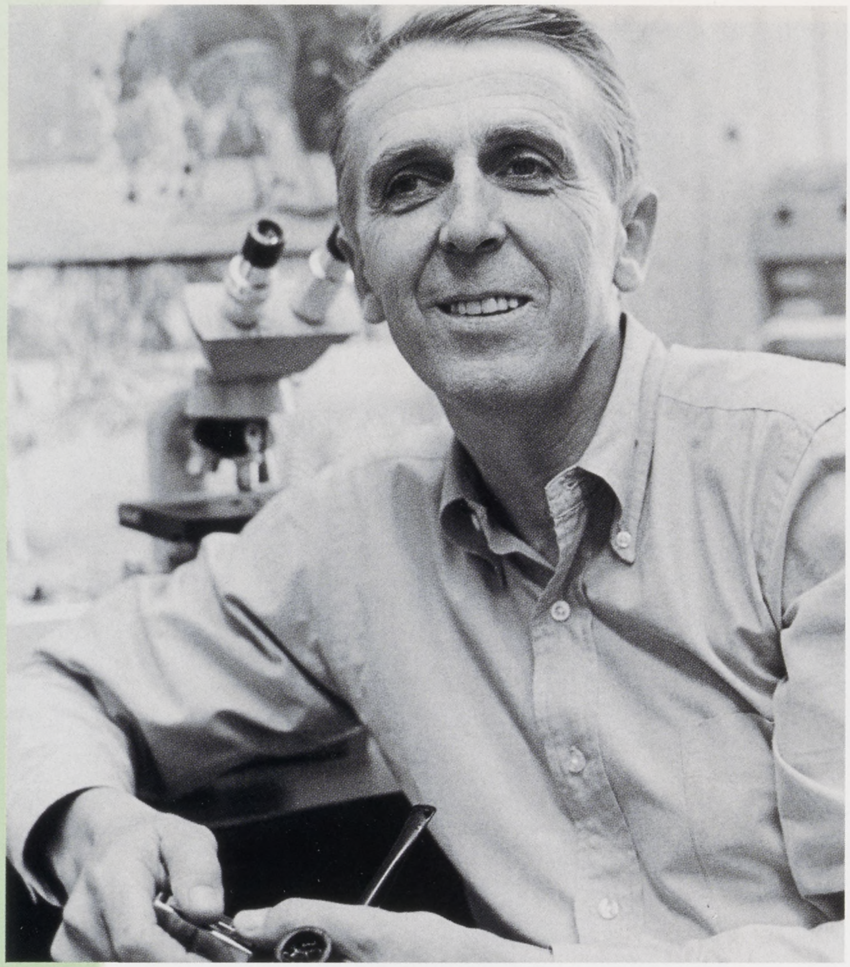
Field studies are usually limited to observation, without much opportunity to do controlled scientific work. An exception is a project currently under way at the comparative physiology's lizard research facility and at a field site in Cuba. It is a test of headstarting, a possible method to preserve the world's most endan-

▲ The Zoological Society of San Diego established an animal research committee as early as 1925 and would later conduct a variety of projects from the Zoo's hospital building.

"You must collect things for reasons you don't yet understand."

—Daniel J. Boorstin, Librarian of Congress Emeritus

This quote is posted above the Frozen Zoo.



▲ In 1975, Dr. Kurt Benirschke became the founding director of CRES. A professor of reproductive medicine and pathology at the University of California, San Diego, Dr. Benirschke is a physician and pathologist by training.

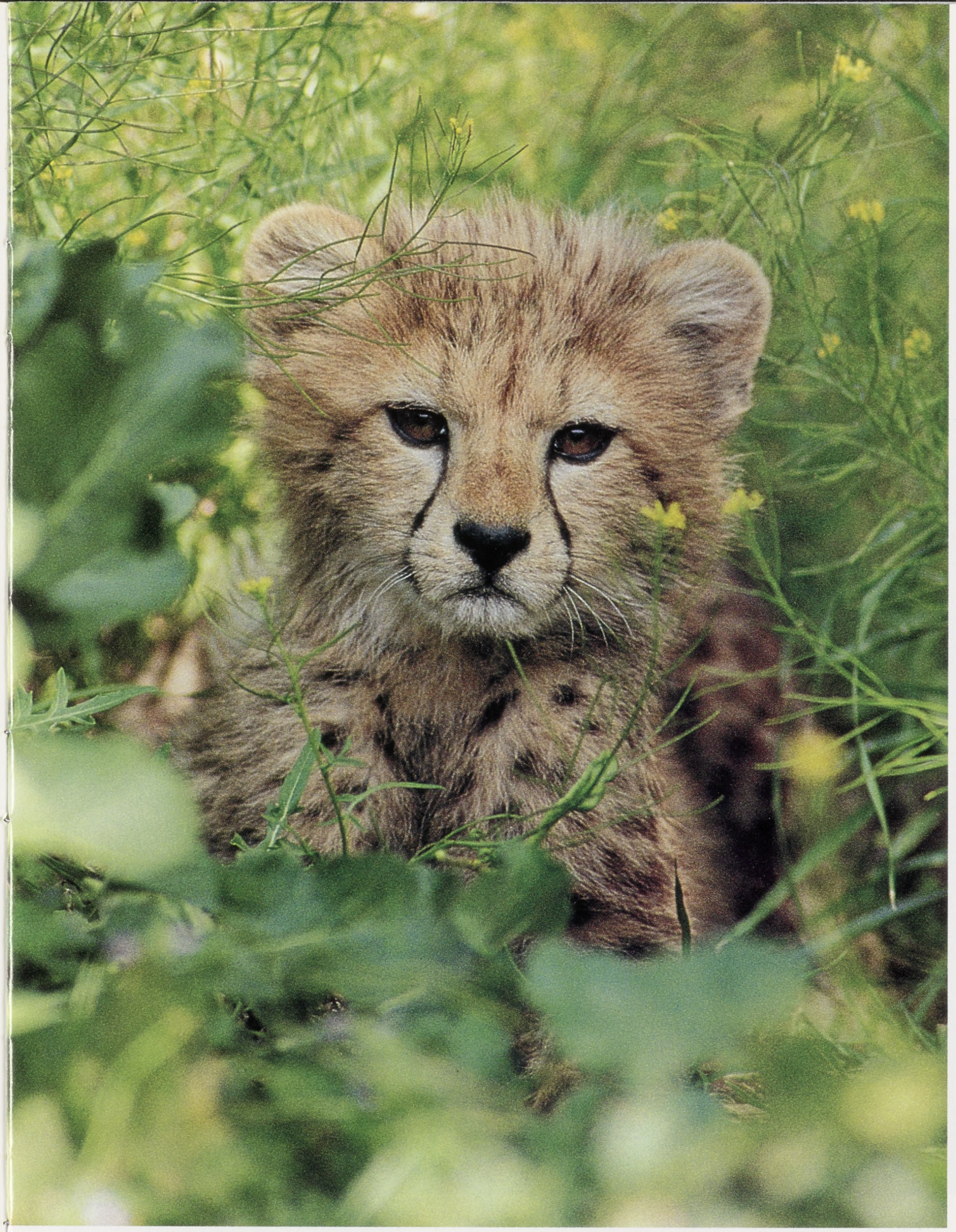
gered lizards: the eight species of giant rock iguanas that live on the islands of the Caribbean. All eight species have had their numbers severely reduced by predators like feral cats, snakes, and birds of prey.

The idea behind the headstarting project is this: suppose hatchlings are raised in captivity until they are a year old, then released. Would more of them live to adulthood, because they were too big for certain predators to eat? It is a question that can be answered by a controlled experiment: release two groups of young lizards simultaneously, some newly hatched and some which have a year's "head start" on their growth. Then evaluate the survival rate of the two groups over several years.

The headstarting experiment is an example of animal modeling. A technique is tested in one species, with the hope that the results will apply to other, similar species as well. In this case, Cuban rock iguanas were selected as the experimental subjects because they are the least endangered of the group. There are 2,000 to 3,000 of the giant lizards on the U.S. Naval Base at Guantanamo, as well as an unknown number on the Cuban mainland and offshore islands. Those numbers are substantial enough to allow for a few eggs to be taken from the wild and hatched experimentally. No one would want to test a new technique on a severely endangered animal like the Jamaican iguana, with a population that may be as small as 50. "You can't do many kinds of studies where the world population is 50 animals; you can't risk it," Dr. Allison Alberts, head of the comparative physiology division, explained.

Field studies usually seem to involve exotic animals in remote locations. An exception is a new project to study the San Diego coast horned lizard. These local native animals are endangered because of loss of habitat—the vanishing ecosystem known as coastal sage scrub. Scientists in the comparative physiology division hope to take what they have learned about exotic lizards and apply it to study and conservation of the native lizard species. An initial study site has been identified in the Temecula area, and other sites will be studied as well. "This is our first purely field project, which does not involve any captive animals at all," Dr. Alberts said.

"I like to mix fieldwork and lab work," said Dr. Valentine Lance, head of the endocrinology division. "The Zoo encourages us to do both, but we usually have to find our own money for fieldwork, through outside grants. For example, I'm doing a project studying desert tortoises on



"In the zoo world we're fairly well known, less so in the academic world. Some academics doubt that zoos are really serious about science. My response is, take a look at what we have done. Anyone who does this with an open mind will be impressed."

—Donald Lindburg, Ph.D., Head of the Behavior Division

◀ The captive breeding of solitary animals, like the cheetah *Acinonyx jubatus jubatus*, has been a constant problem for zoos. Special studies at CRES have focused on this situation, with good results. At present, more than 100 of these cats have been born at the Wild Animal Park.

the military base at Fort Irwin, near Barstow. The U.S. Army is paying for that project. They fund a lot of environmental studies on military bases." The tortoise project focuses on a respiratory disease that has been found to affect 90 percent or more of tortoises in California's deserts. But tortoises on the Fort Irwin base, an area of pristine desert undisturbed by cattle grazing, off-road vehicles, or hunting, appear to be disease free and are currently being tested.

THE NOSE KNOWS

A major focus of study at CRES is the way animals use scent markings to communicate.

It is like trying to learn a new language, one whose richness and importance humans are only beginning to appreciate. CRES scientists look at scent markings from the viewpoints of behavior, physiology, endocrinology, chemistry, and even genetics.

Anyone who has ever walked a dog or held a cat has seen scent marking in action. The dog deposits small amounts of urine in many places and eagerly smells the marks left by other dogs, often covering the other dog's mark with one of its own. The cat rubs its cheek along a person, a chair leg, or a doorway to leave its mark. Scent marks can convey many kinds of information to other animals: an individual's identity, gender, and readiness to mate; territorial claims; possibly even the individual's age and dominance status. A material which carries a scent for communication purposes is called a pheromone.

Scent marks often consist of urine or feces. In addition to these, many animals develop specialized glands whose whole purpose is to secrete scent for communication. Large lizards like iguanas have rows of pores on their legs that secrete a thick material like toothpaste. They deposit it in their environment simply by walking along. The material is not very volatile, so rather than smelling it, other lizards "taste" it by flicking their tongues.

The giant panda secretes a liquid from a scent gland under its tail, rubbing it onto trees for other pandas to find. It calls attention to the marks by clawing the tree. Scent marking is a major focus of CRES research on giant pandas. The behavior division is interested in how the pandas deposit the marks and how they react to them. Endocrinology will examine the relationship between hormonal changes and the chemical composition of scent marks. Compara-

▼Detecting when an animal is pregnant can often be difficult. For the white rhino *Ceratotherium simum simum*, a urinary hormonal assay, which measures hormones like estrogen and progesterone, can be done.

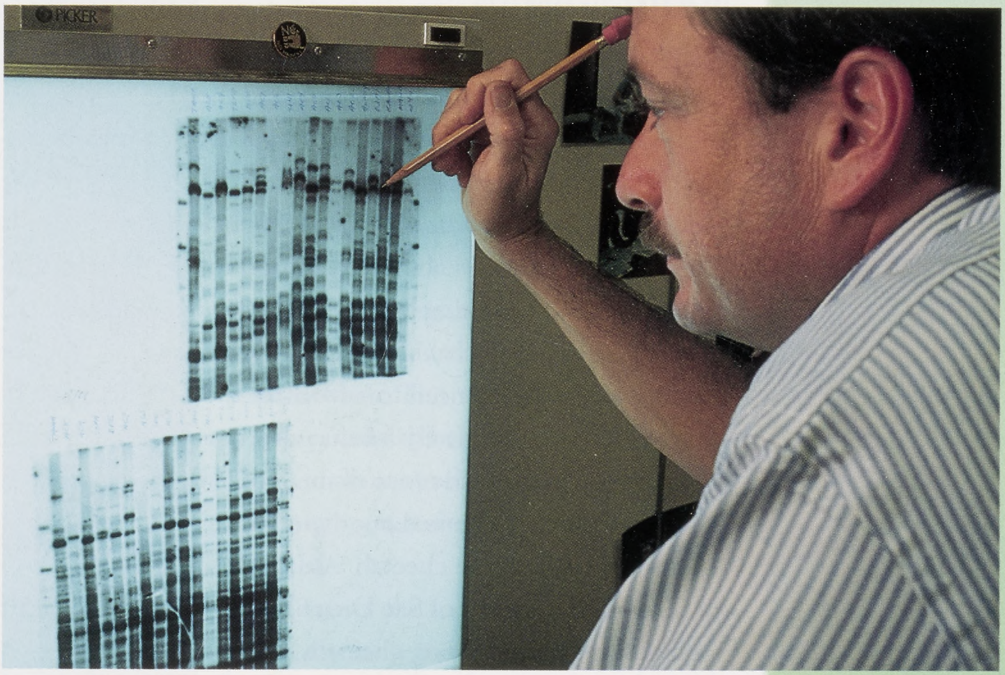
tive physiology and genetics will collect the marking liquid and analyze it in the laboratory; it may give clues to the animal's gender, sexual maturity, and readiness to reproduce. It may even be possible to distinguish each individual's scent markings chemically or genetically. Since the scent-marking material can be collected noninvasively, without disturbing the animal, techniques like these could be extremely valuable for studying wild, free-ranging animals.

ONE'S COMPANY

The traditional method for getting exotic animals to breed was very simple: put a male and one or more females in the same enclosure, then stand back and "let nature take its course." If offspring resulted, fine. If not, there was not much that keepers and curators could do about it.

This approach works just fine for some animals. CRES has a colony of lion-tailed macaques, obtained from His Serene Highness The Prince of Monaco, which has reproduced and sustained itself as a group for decades. But some animals—species as diverse as giant pandas, cheetahs, and monitor lizards—are notoriously hard to breed in captivity. A possible reason came to light as more became known about the behavior of these animals in the wild. In each of these cases, the animal's natural lifestyle was solitary; they might go days or weeks without seeing another of their kind.

Researchers began to suspect that poor captive-breeding results were sometimes caused by unnatural social groupings in the exhibit. A mated pair, a harem-style arrangement, or a breeding group may be appropriate for many species, but it may be all wrong for others. Perhaps the continued presence of the male causes so much stress to the female that she fails to go through normal reproductive cycles. Or perhaps the constant presence of the female makes the male lose interest in her as a potential mate.



▲ Utilizing DNA techniques that were first developed for criminal investigations, CRES scientists, such as geneticist Dr. Oliver Ryder, can study the relationships among individual animals by using only a drop of blood or a sample of the animal's hair.

The breeding of solitary animals, a perennial problem for zoos, is a special focus of CRES research. Behaviorist Dr. Donald Lindburg and his associates have become authorities on breeding solitary species in captivity. "We shifted focus from the social primates—like the macaques—to more solitary species—like cheetahs, giant pandas, and rhinos," Dr. Lindburg said. "A lot of the solitary species are problems for zoos. The animals have their own special requirements. You have to enable the male and female to use normal communication processes to recognize each other."

The institution's wide-ranging expertise permits a broad outlook into the breeding behavior of many different species. "For example, pygmy lorises, cheetahs, and lesser pandas are all solitary species," said Dr. John Phillips. "You could compare their behavior patterns and try to analyze the behavioral system. Is there a common link? Could it be extended to other solitary ani-

> *"The beauty and genius of a work of art may be reconceived, though its first material expression be destroyed; a vanished harmony may yet again inspire the composer; but when the last individual of a race of living beings breathes no more, another heaven and another earth must pass before such a one can be again."*
>
> *—William Beebe, American naturalist and explorer (1877-1962)*

mals, like savanna monitors? Each of these solitary animals is a puzzle—when to put them together, when to keep them apart."

BEASTLY BEHAVIOR

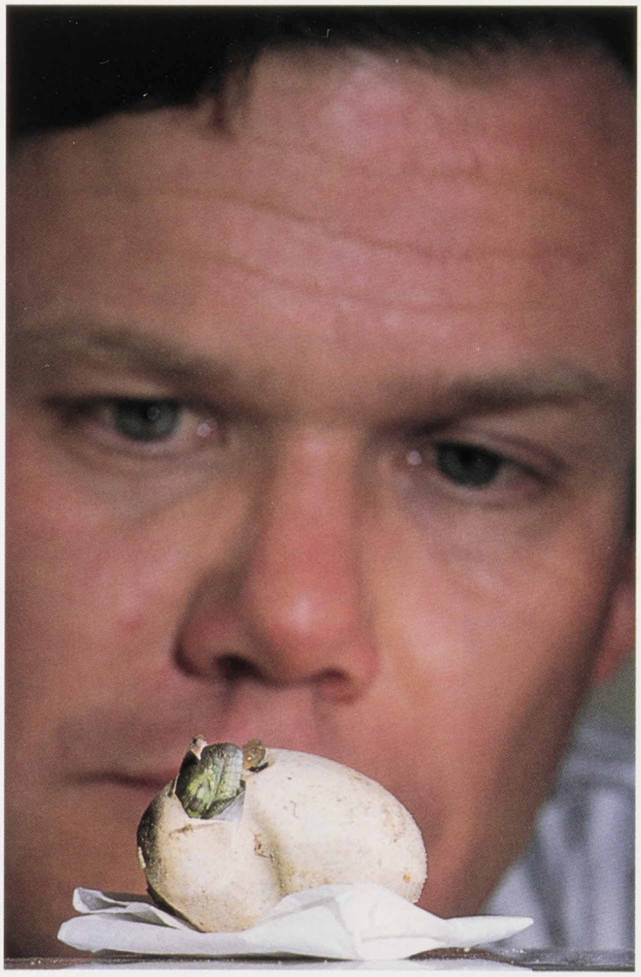
In trying to get endangered animals to reproduce, too little attention is usually paid to the behavior of the animals, Dr. Donald Lindburg says. Head of the behavior division of CRES, he believes that understanding the natural behavior of animals in the wild is absolutely critical to conserving them, and that behavior study is the trend of the future. "Behavior is absolutely basic and fundamental. The need to have male and female procreate is basically a behavioral issue."

Behavior may lie at the root of the problem for many animals that breed poorly in zoos. A classic example is the cheetah. Although the Zoological Society of San Diego has had notable success in breeding cheetahs, with more than 100 cubs born at the Wild Animal Park, most zoos have not done so well. Only a small number of captive cheetahs ever reproduce. Some scientists have concluded that the problem lies in the cheetahs themselves. A widely reported scientific study claimed that cheetahs are simply not very fertile, possibly because of inbreeding. It is true that cheetah males, both wild and captive, have what appears to be poor quality sperm. But CRES research has shown that this "sub-fertile" sperm does not stop cheetahs from breeding when their behavioral needs are met.

Over a 10-year study at the San Diego Wild Animal Park, in which the animals' behavioral cues were carefully followed, 13 male cheetahs were given the opportunity to breed, and 11 of them sired offspring—a much better record than the 15 to 20 percent success rate of cheetah breeding programs nationwide. The fertile males had the same poor quality sperm as had been reported for other cheetahs. "They all have bad sperm, but it's good enough," Dr. Lindburg concluded. "Cheetahs breed fine in the wild and they can breed fine in zoos. When there are problems, it's not the animals' fault, it's ours."

Why do so few captive cheetahs breed? It may be a matter of understanding the life-style of the animal. Cheetahs in the wild are solitary animals that rarely see another of their species. Males and females come together only to breed. In most zoos, the animals are housed together or in adjacent enclosures. That is an abnormal situation for the cheetah, and it may cause so much stress that it blocks the female's normal reproductive cycle.

When the female is fertile and cycling normally, it is important to choose the proper moment to bring the male and female together. One way of determining the female's readiness



◀ Dr. John Phillips (left) watches the hatching of a green iguana *Iguana iguana*. Due to new captive-breeding efforts, these green iguanas are making a comeback in their native range. Together with comparative physiologist Dr. Allison Alberts (opposite page), Dr. Phillips helped launch a baby boom of green iguanas by mimicking as closely as possible, in a captive setting, the lizard's natural breeding conditions.

is by testing her blood, feces, or urine for hormones like progesterone. A much simpler and less invasive method may be to observe the animal's behavior carefully. The female marks her surroundings with her urine, which carries a distinctive scent. A male cheetah can learn a great deal from these scent marks, including her estrous state. So one way to determine the perfect time for breeding is to allow the male to examine the female's enclosure for a few minutes each day, at a time when she is not there.

"We were impressed by the amount of attention the male paid to scent markings in becoming aroused," Dr. Lindburg said. "If the female is in estrus and he is exposed to her scent markings, he gets the message and his behavior changes dramatically. He begins to vocalize in a distinctive way we call a stutter bark, and he searches the area, running up and down the fence line."

BIOLOGICAL BLUEPRINTS

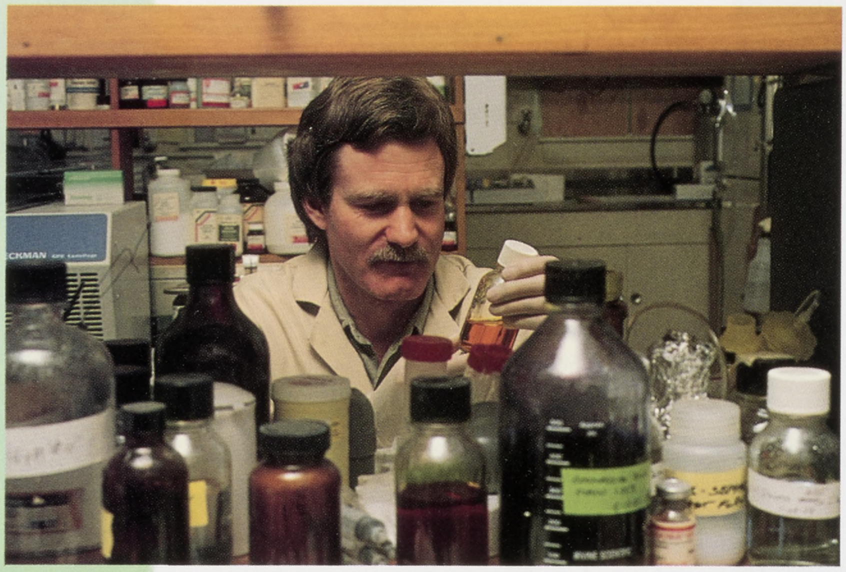
Some of the most basic research at CRES is done by the genetics division, using both cutting-edge DNA technology (molecular genetics) and traditional methods of analyzing chromosomes by their appearance (cytogenetics). Using techniques that were originally developed for criminal investigations, CRES scientists can analyze the relationships among individual animals or species of animals, using only a drop of blood or a sample of the animal's hair. This technique has enormous potential, because it could permit study material to be collected without disturbing or even seeing the animal being studied.

"Some of the most intriguing results from this technique involve gorillas," said geneticist Dr. Oliver Ryder, who has been with CRES almost since its founding. Insights gained at CRES from DNA testing of the various subspecies of gorillas could eventually result in a reclassification of the species. Science currently recognizes three subspecies of gorilla: the mountain gorilla *Gorilla gorilla beringei*, the eastern lowland gorilla *Gorilla gorilla graueri*, and the western lowland gorilla *Gorilla gorilla gorilla*. All are endangered, and only the western lowland gorilla is found in zoos. Samples of blood and hair from western lowland gorilla groups at the Zoo and the Wild Animal Park were used to develop the technique for getting good quality DNA from hair, and to learn the DNA sequences that would allow comparisons of individuals and groups.

As often happens, the project was undertaken to answer a specific question, but it provided results with much broader implications. The specific problem had to do with paternity in groups of mountain gorillas in the wild. Field researchers needed some way of telling which

"You never get tired of seeing the animals. Everybody here is an animal person. There's a lot of emotional as well as intellectual commitment to the animals. Scientists are supposed to be detached, but it's not that way at all."

—Barbara Durrant, Ph.D., Head of the Reproductive Physiology Division



▲The virology and immunology division of CRES, headed by Dr. Michael Worley, continues to study the prevalence of a retrovirus infection in koalas. Since the majority of deaths in captive adult koalas are the result of cancer or opportunistic infections, there is probably a link between these causes and the presence of a retrovirus.

of several adult males in the group was the father of each infant. Since the groups were wild and wary of people, the only medical samples available were hair and feces left behind in the sleeping nests where the group spent the night.

"Given an infant with five possible fathers, we were able to study the DNA of the baby and the adult males, and then use the results to rule out certain males from being the father," Dr. Ryder said. "Ultimately to assign paternity, you need to have samples from all potential fathers and be able to exclude all but one." It is usually assumed that the dominant male gorilla in a group is the father of all the infants, but the results showed that this was not true in all cases. Sometimes it turned out that one of the subordinate males had succeeded in breeding.

Since publishing this study, CRES scientists have received requests for paternity studies from several other zoos, involving groups of gorillas, orangutans, and slow lorises. Dr. Ryder observes that "We were able to resolve those paternity issues clearly. In some cases the studbooks will need to be corrected. It's important that the pedigrees of captive animals be correct, because it affects breeding assignments designed to preserve the gene pool and control inbreeding."

Management of captive breeding populations of endangered animals requires that two mated individuals are actually of the same species. Often, species and subspecies identity cannot be determined by morphology (physical characteristics) alone. One objective of the CRES cytogenetics division is to evaluate the chromosomal makeup of animals for species or subspecies identification.

For example, in the case of Kirk's dik-dik, a diminutive antelope of sub-Saharan Africa, both captive and wild populations from different regions were found to have highly divergent

as humanly possible. The answer had to be available within 36 hours so that the bird could be released.

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HOW YOU CAN HELP

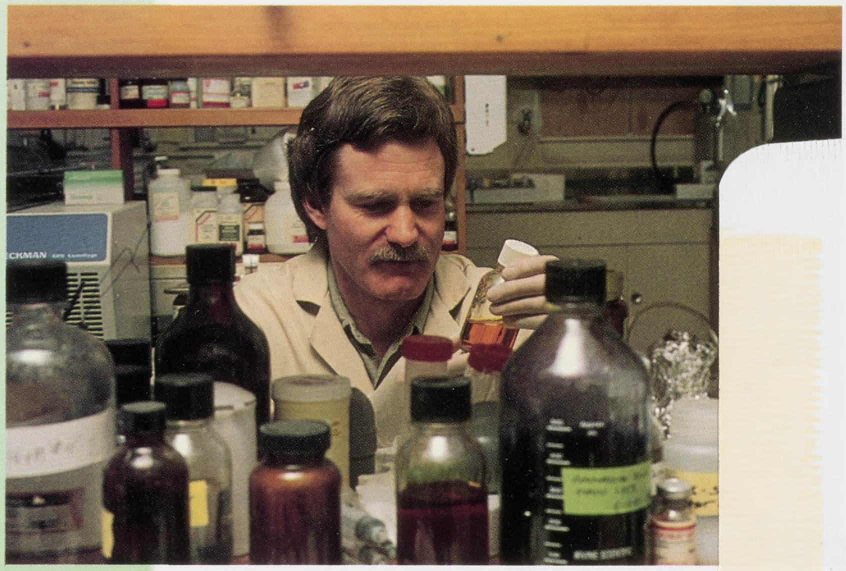
More than $2,500,000 each year is required by the Center for Reproduction of Endangered Species to save rare and threatened animals. The principal source of funding for CRES is the Zoological Society of San Diego, but more than $1,000,000 each year is granted to CRES from CRES endowment income, foundations, corporations, government agencies, and hundreds of individuals concerned with the continued existence of wildlife.

On a scientific level, those funds have generated more than 1,000 scientific papers and technical reports, led to various basic and applied breakthroughs, and allowed for the continued pursuit of research excellence. Several hundred students, from undergraduate to postdoctoral levels, have received training in conservation through internships at CRES. Recently, the National Science Foundation, the most prestigious, peer-reviewed scientific agency in the United States, has rated science at CRES in the top 10 percent of all institutions nationwide.

CRES continues to strive for financial independence through its endowment fund, which increases through the generosity of caring friends like you. About one-third of our $35,000,000 long-term goal has been reached. Please help us make this goal a reality by sending a gift to CRES in the envelope provided in this issue. Your support of our conservation and research efforts is most appreciated.

For additional CRES giving opportunities, please call Amy Parrott in the Development Office at (619) 685-3213.

"Given an infant with five possible fathers, we were able to study the DNA of the baby and the adult males



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in the sleeping nests where the group spent the night.

For example,

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More than $2,500,000 each year is required by the Center for Reproduction of Endangered Species to save rare and threatened animals. The principal source of funding for CRES is the Zoological Society of San Diego, but more than $1,000,000 each year is granted to CRES from CRES endowment income, foundations, corporations, government agencies, and hundreds of individuals concerned with the continued existence of wildlife.

On a scientific level, those funds have generated more than 1,000 scientific papers and technical reports, led to various basic and applied breakthroughs, and allowed for the continued pursuit of research excellence. Several hundred students, from undergraduate to postdoctoral levels, have received training in conservation through internships at CRES. Recently, the National Science Foundation, the most prestigious, peer-reviewed scientific agency in the United States, has rated science at CRES in the top 10 percent of all institutions nationwide.

CRES continues to strive for financial independence through its endowment fund, which increases through the generosity of caring friends like you. About one-third of our $35,000,000 long-term goal has been reached. Please help us make this goal a reality by sending a gift to CRES in the envelope provided in this issue. Your support of our conservation and research efforts is most appreciated.

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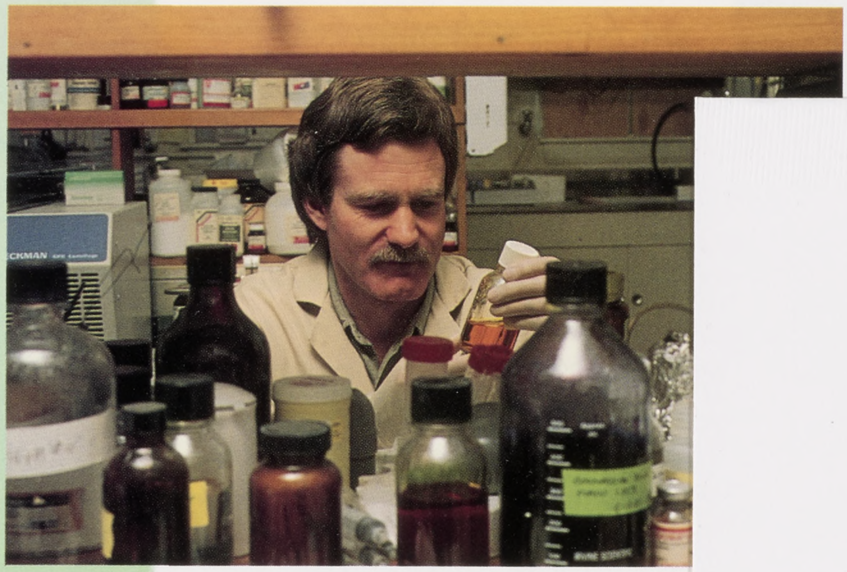

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diminutive antelope of sub-Saharan Africa, both captive and wild populations from different regions were found to have highly divergent chromosomal makeups. These animals appear physically alike, but matings between members of the different genetic populations produce sterile offspring.

Arlene Kumamoto and Steve Kingswood, cytogenetic researchers at CRES, have identified four different chromosomal populations of Kirk's dik-dik. If there is to be successful management of captive animals, then genetic compatibility must be determined. In the wild, field biologists often resort to translocating animals in order to establish or augment existing populations. Understanding genetic variability within a species is an important consideration for wildlife management plans, both in captivity and in the wild.

One of CRES's assignments involved the California condor. When CRES was founded, fewer than 30 condors remained in the wild, and very little was known about them. Studying condor behavior, zoo curators and field biologists, working together on the Condor Recovery Team, needed to know details about each bird, starting with its gender. But the sexes are indistinguishable except by surgical laparoscopy, genetic testing, or hormonal analysis of the droppings—which can only be done on adults.

At first, the California Department of Fish and Game had a program of trapping, studying, and releasing the condors. The decision whether to release them depended in part on whether they were male or female. The cytogenetics laboratory at CRES was asked to help answer that question. So whenever a wild condor was trapped, a few milliliters of blood were drawn and rushed to San Diego. The slow, labor-intensive process of chromosome analysis was begun immediately and carried out by CRES cytogenetics specialist Arlene Kumamoto, working day and night to get the results as fast as humanly possible. The answer had to be available within 36 hours so that the bird could be released.

As the condor population continued to fall, a captive-breeding program was begun, first with wild-laid eggs, later with captive pairs of condors. The CRES genetics division was called upon again, this time to determine the relationships among the condors so that breeding pairs could be set up. "We did DNA studies and found they all belonged to one of three clans, like a nuclear family in humans. We knew we were studying the entire variation of the species—this is all the animals that can have any descendents. They are now incorporated into a breeding plan designed to preserve as much of their total genetic material as possible," Dr. Ryder said.

Captive-breeding programs at the Los Angeles Zoo and the San Diego Wild Animal Park succeeded in quadrupling the world population of California condors. CRES still plays a role, determining whether each hatchling is male or female. New technology has replaced the time-consuming cytogenetic studies. CRES researcher Leona Chemnick uses a DNA probe that can identify a genetic sequence present in female condors but not in males. The older method required several milliliters of blood, which in turn required waiting until the chick was several months old. The

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DNA probe technique can be used on a single drop of blood. Now Leona Chemnick is testing a method that uses residual blood in the eggshell to determine the chick's gender. This has numerous advantages: it requires no handling of the animal and is completely noninvasive (no blood drawing or tissue samples are needed), and it can be done as soon as the chick is hatched.

▲Field studies that include the examination of urine and fecal samples have assisted researchers in understanding the reason for lower birth rates between populations of the captive western lowland gorilla *Gorilla gorilla gorilla* (above) and the free-ranging mountain gorilla *Gorilla gorilla beringei*.

SEEDS OF LIFE

Dr. Barbara Durrant, head of the reproductive physiology division, shares a tiny office with a technician, a postdoctoral fellow, and a number of students. The office hums constantly with the sounds of cryopreservation. In the adjacent locked room, two large tanks of liquid nitrogen hold bits of germ plasm (sperm, eggs, and embryos) that are part of the best-known feature of CRES: the Frozen Zoo, a cell bank sometimes called the Twentieth-Century Ark. The Frozen Zoo is like a genetic bank vault, an archive of irreplaceable genetic information on hundreds of rare and endangered species.

The Frozen Zoo was originally the brainchild of Dr. Kurt Benirschke, founding director of CRES. Early in his career, Dr. Benirschke rejected the distinction between human and veterinary medicine. "I feel strongly that most biological problems are very similar, with some variations," he stated. "Things like placentation, endocrinology, viral infection—these are very similar from one species to another." This belief fostered a career in which he moved freely back and forth between human medicine and zoological science. While consulting for a New York game farm, he began collecting tissue samples from a variety of exotic and endangered animals. When he became director of research at the San Diego Zoo in 1975, he already had a sizable cell bank collection that formed the nucleus of the Frozen Zoo.

Dr. Durrant and her colleagues do much more than just preside over the collection of germ plasm in the Frozen Zoo. The reproductive physiology division is also responsible for artificial insemination, embryo transfer, hormonal stimulation, and all the other methods for obtaining offspring from animals that cannot quite manage to reproduce on their own. Many of the techniques are adapted from the huge and well-funded field of human infertility research.

An early coup for CRES was the first Chinese monal pheasants hatched by artificial insemination. The Zoo had three monals, a beautiful and endangered pheasant, but neither of the females was able to breed normally. One had an injured leg that made her unable to bear the male's weight, and the other was in isolation because she had been exposed to a contagious disease. Artificial insemination seemed to be the answer, but it had never been done in this species. Using what was known about such techniques for other birds, CRES scientists managed to inseminate both females, and three chicks were produced—doubling the population of the birds outside China.

▲ The pathology laboratory, headed by Dr. Linda Lowenstine, is a part of the CRES team. Studies completed here help living animals lead better and healthier lives. This is one of the largest zoo pathology departments in the world.

In this case fresh semen was used, but Dr. Durrant and her group realized that it would be valuable to be able to inseminate pheasants with refrigerated or frozen semen. In cooperation with the Zoo's bird department, they perfected their techniques by studying two less endangered Chinese pheasants, the Himalayan monal and the Temminck's tragopan. "We got a 75 percent hatch rate using refrigerated semen and a 50 percent hatch rate with frozen semen," Dr. Durrant said. "That compares very favorably with the best results achieved with any bird species."

In many cases, the focus of the reproductive physiology department is on perfecting a technique rather than achieving a particular birth or hatching. "The major focus of all our basic research is germ plasm preservation. We want to know the best ways to collect, prepare, freeze, and thaw the genetic material. Our applied research has to do with what happens after it is thawed."

Why so much emphasis on freezing and preserving? These techniques permit the mating of animals that cannot physically come together, because of geographic separation or even separation in time. For example, a genetically valuable male cheetah could continue to sire offspring long after his death. Inbreeding—the bane of captive-breeding programs—can be avoided by shipping frozen sperm across countries or continents.

SOLVING REPRODUCTION PUZZLES

As the name Center for Reproduction of Endangered Species implies, propagating rare animals is a major focus of CRES. Heavily involved in that campaign is the endocrinology division, which has been part of CRES since its founding 20 years ago, along with the genetics

▲ An early CRES project involved Darwin's rheas *Pterocnemia pennata pennata*. At that time, the Zoological Society of San Diego maintained the only breeding colony of these birds in the country, but infant mortality was excessive. Bacterial infection was determined to be the young birds' main problem and was treated.

and reproductive physiology divisions. "At that time, no other zoo had anything like what we were starting," endocrinology specialist Nancy Czekala recalled. "It was a wide-open field. Hormonal analysis was needed to answer so many questions. Is this animal ovulating? Why isn't she getting pregnant? We needed laboratory methods to detect pregnancy and predict the time of birth."

CRES gained international recognition early in its existence for developing techniques to measure hormones noninvasively—that is, without the need to capture the animal and draw a blood sample. One such technique, developed at CRES and now in standard use throughout the world, uses bird droppings to determine whether a particular bird is male or female—a critical piece of information but sometimes not apparent from the bird's appearance. "What sex is this animal?" is still a frequently asked question that is crucial to successful reproduction in captivity.

Trying to study an animal's intimate chemistry without disturbing the animal, CRES researchers may work with all kinds of startling specimens. "I have freezers full of freeze-dried rhinoceros dung," Nancy Czekala remarked. "I've been working with a ranch in Zimbabwe that has free-ranging black rhinos. I want to see if their hormonal cycling is the same as it is in captive rhinos. They collected fecal samples from 20 rhinos every day for a full year. It could take several years to analyze them all."

Another frequent assignment for the endocrinology division is detecting when an animal is pregnant. Hormones like estrogen and progesterone can be measured in urine specimens for many species. On one memorable field project, Nancy Czekala found herself in Rwanda, following female mountain gorillas around to obtain urine samples.

It was also discovered that a urinary hormonal assay for pregnancy detection can be done for Indian rhinos but not for black rhinos. Eventually, Nancy Czekala determined that saliva samples would work and collected them from black rhinos at the Zoo and the Wild Animal Park. How do you get a saliva sample from a rhinoceros? "It wasn't too hard. You hold up a carrot, the rhino salivates, and you withdraw the saliva sample from her mouth using a syringe with no needle."

LIVING LONGER, LIVING BETTER

One of the missions of CRES has always been to improve the health and well-being of animals at the Zoo and the Wild Animal Park—

and, by extension, of animals everywhere. In 1975, the dust from moving in had scarcely settled when the fledgling research department was presented with its first challenge. As would happen repeatedly over the years, the challenge arose from an acute need for information about animals in the Zoo's collection.

According to Dr. Benirschke, "One of the earliest things that happened was that the late K. C. Lint, then curator of birds, came to us and said, 'I'm so glad you're here—we need help curing the Darwin's rheas.' We had the only breeding group of these South American birds in the country, perhaps in the world, and the species was severely threatened. Although our birds were laying eggs and hatching chicks, the infant mortality was excessive."

The staff quickly determined that the main problem confronting the young chicks was bacterial infection, mostly of the *Salmonella* type. The next problem was to figure out a way to prevent or cure the infections. "We came up with solutions. We started hatching Darwin's rheas and raising them ourselves, upstairs. We brought in a meat grinder to prepare their food and raised them on artificial turf. The first group that hatched that year had had 100 percent mortality. By the end of the season, the final clutch of eggs had 100 percent survival." The work resulted in a lengthy scientific paper about the endangered birds, identifying health hazards and how to correct them. It also resulted in a number of healthy, disease-free young birds that settled at the Wild Animal Park.

Animal health is a particular focus of the infectious diseases, virology, and pathology divisions. While carrying out basic research as time permits, these divisions do a lot of service work: laboratory testing for the presence of pathogens or disease. This work is vitally important to the veterinarians at the Zoo and the Park. "I believe viral diseases are a significant problem in zoos," said Dr. Michael Worley, head of the virology and immunology division. "Many infectious diseases are more of a problem in captivity because of population density, unnatural housing and, perhaps, inappropriate nutrition and stress."

Virologists at CRES use the latest methods borrowed from human medicine: isolating, cloning, and replicating the DNA of the virus, then developing a polymerase chain reaction (PCR) test for the presence of that specific virus. Although there is no treatment for most viral diseases, sometimes the test alone can result in dramatic improvements in the health of the collection.

"Right now the world has the most genetic material it's ever going to have. Less than yesterday, more than tomorrow. The most important thing is to get that genetic material into the bank."

—Barbara Durrant, Ph.D., Head of the Reproductive Physiology Division

◀One of the most devastating viral infections in zoos, as well as in the wild, is malignant catarrhal fever (MCF). The rare Père David's deer *Elaphurus davidianus* is so susceptible to MCF that entire herds have been wiped out. CRES scientists continue to study the virus and look for solutions.

Dr. Werner Heuschele, who is head of the infectious diseases division as well as director of CRES, targeted one of the important viral scourges in zoos as well as in the wild: malignant catarrhal fever (MCF). A herpes-type virus, it has devastated populations of deer, cattle, bison, buffalo, and antelope in many zoos. Père David's deer, a rare species that has been extinct in the wild for approximately 2,000 years, is so susceptible to MCF that entire herds have been wiped out. The virus is transmitted by "carrier" animals, which are infected by the virus but remain healthy. Carriers include all species of wildebeest as well as sheep and goats, both wild and domestic.

▶ Behavior specialist Helena Fitch-Snyder (left) and Dr. Barbara Durrant, head of the reproductive physiology division, take a swab from a slow loris. Dr. Durrant's division is responsible for using techniques such as embryo implants, hormonal stimulation, and other methods for obtaining offspring from animals that have difficulty reproducing on their own.

> *"People tell me the animals would be better off in the wild, and I say, 'what wild?'"*
>
> *—Werner Heuschele, D.V.M., Ph.D., Director of CRES*

After severe outbreaks of MCF at the Wild Animal Park in the early 1980s, Dr. Heuschele began testing for the presence of the virus in carrier animals in zoos throughout the country. "We found that most of the wildebeest at the Wild Animal Park had the virus, but there were animals at other zoos that were negative. We suggested the curators work at acquiring uninfected animals. By 1984, we succeeded in establishing a totally MCF-free herd of wildebeest." The virus is still present in some species of wild sheep and goats at the Wild Animal Park, but enclosures were rearranged so that they could not infect adjoining groups of deer and other susceptible animals.

Infectious disease research can be very helpful in clinical management. For example, hoofed animals that are being hand raised for any reason are very susceptible to severe and often fatal diarrhea, caused by organisms in their surroundings. Dr. Heuschele suspected that if the babies could have access to colostrum—the first milk given by the mother in the early days after birth—they might do better because of protective antibodies present in the colostrum. Veterinarians arranged to obtain colostrum from commercial dairies, which was then pasteurized. Hand-raised hoofstock babies received 10 percent colostrum in their feedings during their first 3 weeks of life. The results were dramatic: the incidence of fatal diarrhea in newborns was cut in half.

A good deal of the virology division's time is also spent on service work: diagnostic testing for the presence of viruses in specific animals, at the request of staff veterinarians. But there is time for more basic and exploratory study. The information gained can be vital in making management decisions about the animals. For example, Dr. Worley discovered that many koalas at the Zoo, as well as in the wild, were infected with a retrovirus—which could explain why several koalas here have developed lymphatic cancers like lymphoma and leukemia. He succeeded in isolating the virus and developing a PCR test for it. That test is already being used to control the spread of the virus between zoos.

"For example, there was a male who was going to be sent to another zoo for breeding purposes. But we found he was infected with the retrovirus, and the female at the other zoo wasn't. Based on this information, it was decided not to send him there," Dr. Worley said.

He has since found a similar retrovirus in several other species of Australasian marsupials, including the cuscus, potoroo, and wallaby. "This is important because Australia has had a problem with extinction, with many native fauna currently on the brink of vanishing. This might be a contributing factor to the decline of some of those species. We'd like to develop a broad-based, cross-species test and then transfer the technology to virologists in Australia."

The pathology division, headed by Dr. Linda Lowenstine, examines the causes, progression, and consequences of disease. The knowledge gained is integral to the successful management and health of both captive and free-living animals. In his book, *The Pathology of Zoo Animals* (1983), Dr. Lynn Griner reviewed the results of necropsies he conducted during his 14-year tenure as the Zoo's pathologist. His data allowed clinical veterinarians and animal managers to improve diets, housing, and medical procedures for the betterment of captive animal health. CRES pathologists continue to improve upon this data base and have identified many problems in need of further investigations, both here and at other institutions. Dr. Lowenstine and associate pathologists Dr. Bruce Rideout and Dr. Ilse Stalis are adapting modern diagnostic techniques, such as immunohistochemistry, for use in captive and wild animals. No detail is considered to be too trivial.

Wild populations have also benefited from the pathology division's work. Studies of California condors examined the often fatal effects of this scavenger's natural behavior in man-made environments. Causes of death are also being examined for endangered populations of San Clemente Island loggerhead shrikes,

◀ Committed to helping save the giant panda *Ailuropoda melanoleuca* from extinction, the Zoological Society and CRES are working alongside Chinese officials and researchers to increase the numbers of these appealing animals in the wild. Below: On a recent visit to China, CRES endocrinology specialist Nancy Czekala observes a panda at the Xian Zoo. As new techniques are developed, the information is being sent along to scientists in panda breeding centers within China.

"What we are about is strategic planning in conservation, linking the efforts of zoos together into a coherent and holistic conservation plan. It's important to try to link zoo conservation with conservation of wildlife in its habitat."

—Oliver Ryder, Ph.D., Head of the Genetics Division

Hawaiian forest birds, bighorn sheep, and mountain gorillas, as well as populations of sea otters, harbor and elephant seals, and California sea lions.

CRES pathologists collaborate with numerous outside investigators, museums, and institutions. For example, the forensics laboratory of the U.S. Fish and Wildlife Service is responsible for developing morphological and molecular techniques to identify illegally obtained animal products. Illegal traffic in endangered species will only be halted if the consumers and suppliers can be successfully prosecuted through the use of scientific data. The Society's pathology division has received a certificate of merit for its help in this international effort.

▲ Perhaps the best-known feature of CRES is its Frozen Zoo—a cell bank that represents an irreplaceable genetic archive for hundreds of rare and endangered species. This genetic bank vault was the brainchild of CRES founding director Dr. Kurt Benirschke. Arlene Kumamoto, cytogenetics specialist, is holding a number of vials near the cryogenic freezer.

ALL FOR ONE

One thing that makes CRES unique is the presence of so many scientists working in different disciplines. When they all bring their expertise to bear on a single species, the results can be impressive. An example of the multidisciplinary approach is rhinoceros research, particularly studies of the black rhino. Unlike the southern white rhinos, black rhinos have not bred well in captivity. Ongoing rhino projects involve many CRES divisions, including behavior, endocrinology, virology, reproductive physiology, and genetics.

In a remarkable example of cross-disciplinary work, virologist Dr. Michael Worley is currently studying a possible genetic defect in black rhinos. It began as a study of a serious hemolytic disease that affects captive rhinos. He started out looking for viral causes, but his colleagues eventually concluded that the real problem might be a deficiency of catalase, one of the blood enzymes that protect red blood cells from damage. Using molecular techniques, he created a DNA probe that tests for the presence of the catalase gene. Now he is using it to test black rhinos in many institutions. "We're hoping we can develop molecular reagents to detect genetic variation and determine which rhinos do not have the enzyme deficiency. We are slowly going to develop the ability to manage this disease. The fear is that, because of the global endangered status of the black rhino, it may be too slow."

Chromosomal studies of black rhinos undertaken at CRES by Marlys Houck have shown that black rhinos are not genetically uniform throughout their range. East African and South African black rhinos can be distinguished by details of chromosome structure. Knowledge of differences between regional subspecies is important for conserving rhino populations and the species gene pool.

Because of his expertise in breeding solitary species, the AZA Rhino Advisory Group asked Dr. Lindburg to promote studies of all the species of rhinos in North America. Captive rhinos are not reproducing at a self-sustaining rate. One problem may be that rhino courtship is very rough. Often keepers are reluctant to let it proceed, for fear that one of the animals might be injured. "We really haven't done a behavior study of rhino courtship," Dr. Lindburg said. "We will try to enlist keepers and curators at various institutions to videotape rhino pairings and matings. Then we will study the tapes for insight into what works and what doesn't. A critical variable may be adequate space, so the female can avoid being hurt. Other variables may be the temperament of individuals and whether the male has had adequate opportunity to switch from territorial defense to courtship."

Several divisions are involved in studying the reproductive systems of rhinos, particularly the hormonal cycling of the females. Endocrinology specialist Nancy Czekala works with samples of feces, urine, and even saliva from rhino females in many institutions and in the wild. Reproductive physiologist Dr. Barbara Durrant is proud of her role in getting two northern white rhinos at the Wild Animal Park to begin normal hormonal cycling. "They were adults, 20 years old, but had never cycled. We did a workup and they seemed healthy, so we treated them with hormones. In effect, we kick-started their cycles, and now they are cycling normally. Now if we can just get the male to breed them, we'll be in business."

▲ CRES, through its reproductive physiology division, achieved a first with the Himalayan monal pheasant *Lophophorus impeyanus* by successfully artificially inseminating a female with frozen semen.

◀ Determining the fertility and development stage of eggs, such as these belonging to the American alligator, is part of the job of endocrinologist Dr. Valentine Lance.

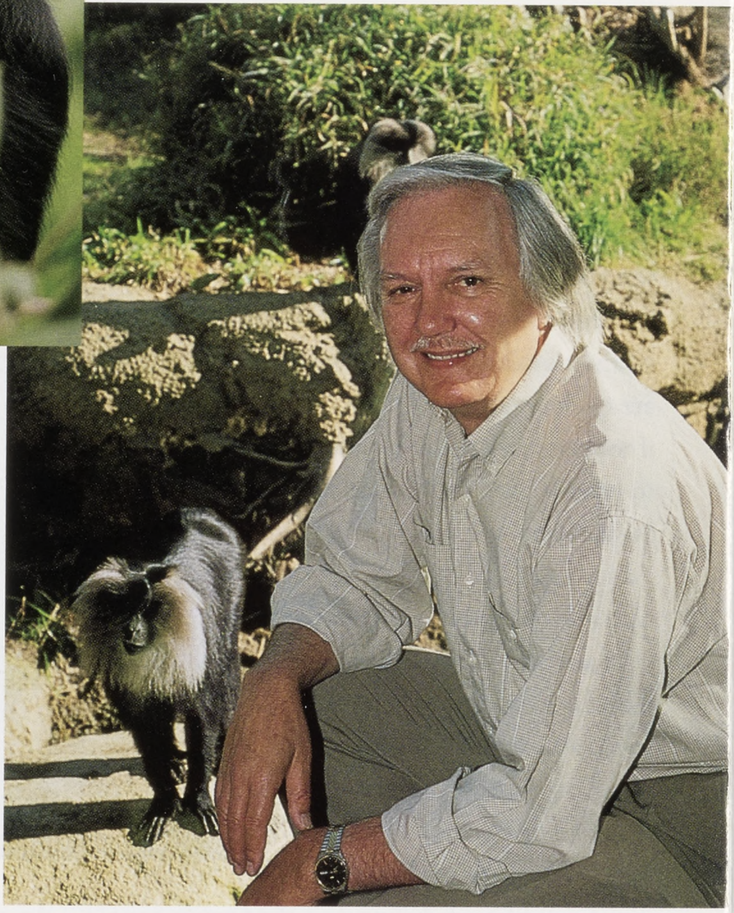
LEADING THE WORLD

In a world where wild habitat is decreasing and some species are rapidly declining in numbers, no zoo and no animal program can exist in isolation. CRES scientists and their colleagues worldwide have taken the lead in coordinating conservation efforts among many institutions and disseminating knowledge that will benefit animals in captivity and in the wild.

Many endangered animals in North American institutions are subject to a Species Survival Plan (SSP) set up by the American Zoo and Aquarium Association (AZA). The SSP committee maintains records of all the animals in North American zoos, keeps studbooks, and makes breeding recommendations. Their recommendations may range from "get as many offspring from this pair as you can" to "don't allow this individual to breed at all." Virtually every scientist at CRES sits on at least one SSP committee; several work with half a dozen or more, their subjects ranging from pandas and orangutans to birds and lizards. Some CRES staffers have

► The endangered lion-tailed macaque *Macaca silenus* has been studied by CRES scientists, especially behavior division head Dr. Donald Lindburg, for well over a decade. In 1983, a colony of lion-tails was obtained by the Zoological Society from His Serene Highness The Prince of Monaco.

► Dr. Donald Lindburg (right) is editor of *Zoo Biology*, a scientific journal for zoo and aquarium scientists. This publication has been produced out of CRES for the past seven years and is now published in affiliation with the American Zoo and Aquarium Association.



additional national responsibilities, such as maintaining a studbook or serving as a species coordinator.

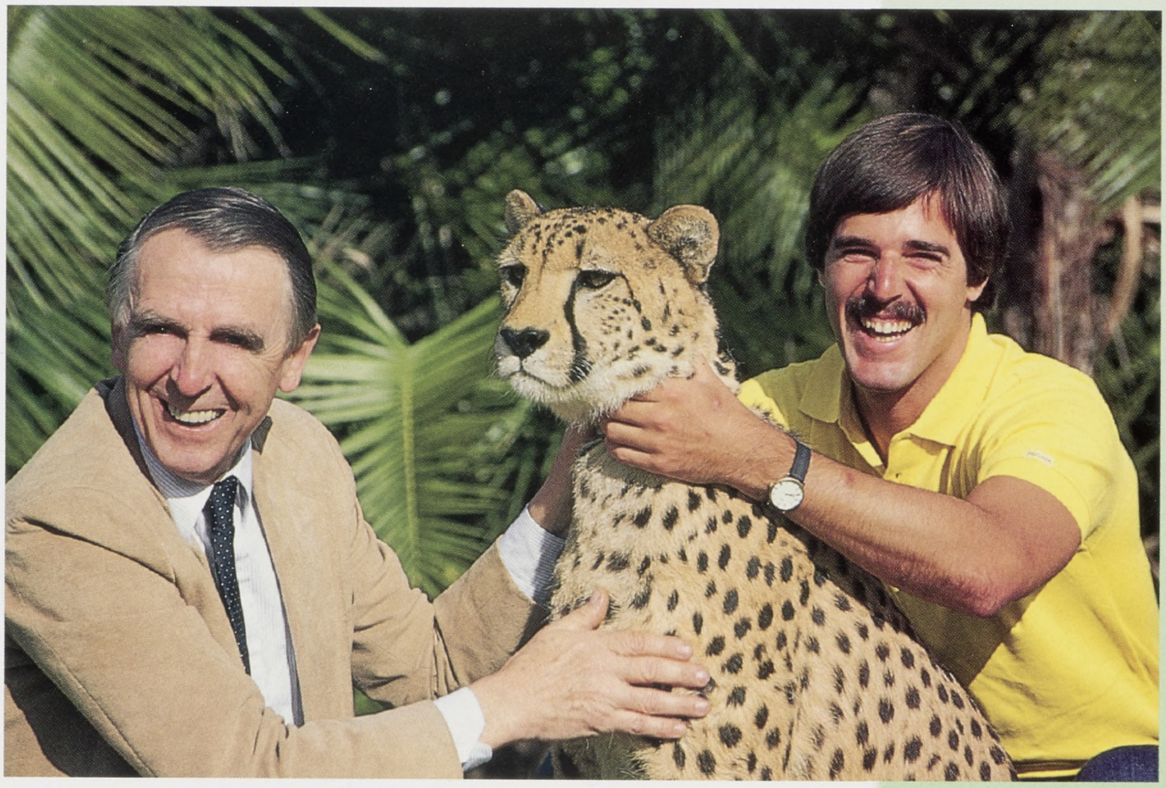
World leadership can also mean leading a multinational consortium to rescue a specific animal. A major effort now under way involves the taguá, or giant peccary, a shy and secretive denizen of the Paraguayan desert, which has only been known to science since the early 1970s. CRES maintains a herd of about 70 of these endangered animals in their native Paraguay. Plans are under way to form two additional herds, one in a Polish zoo that has special expertise in peccaries, and one in the Phoenix Zoo, where the animals' arid habitat can be duplicated.

When research projects are undertaken in other countries, an increasing effort is being made to involve local scientists and students. Efforts to reintroduce animals to the wild or establish preserves inevitably involve other countries, and it is important that the other country be part of the conservation team.

FINDING CASH FOR THE CRITTERS

The year was 1980, the research department was five years old, and it was already obvious that the Zoological Society budget could not possibly pay for everything the scientists were doing. It was and would remain an integral part of the Zoological Society, but the trustees could not provide all the funding. The research department needed some ongoing sources of funding—and perhaps an identity of its own.

It gained both, partly through the talent of Rolf Benirschke, son of Dr. Kurt Benirschke and place-kicker for the San Diego Chargers. He wanted to do something for charity, and since



▲ Kicks for Critters was a fund-raising program begun by Rolf Benirschke, the son of CRES director Dr. Kurt Benirschke. A place-kicker for the San Diego Chargers football team in the 1980s, Rolf asked fans to pledge a certain amount to CRES for each field goal he kicked. Kicks for Critters ended with Rolf's football career, but the awareness has remained. Many current CRES donors are people who first began making gifts to Kicks for Critters.

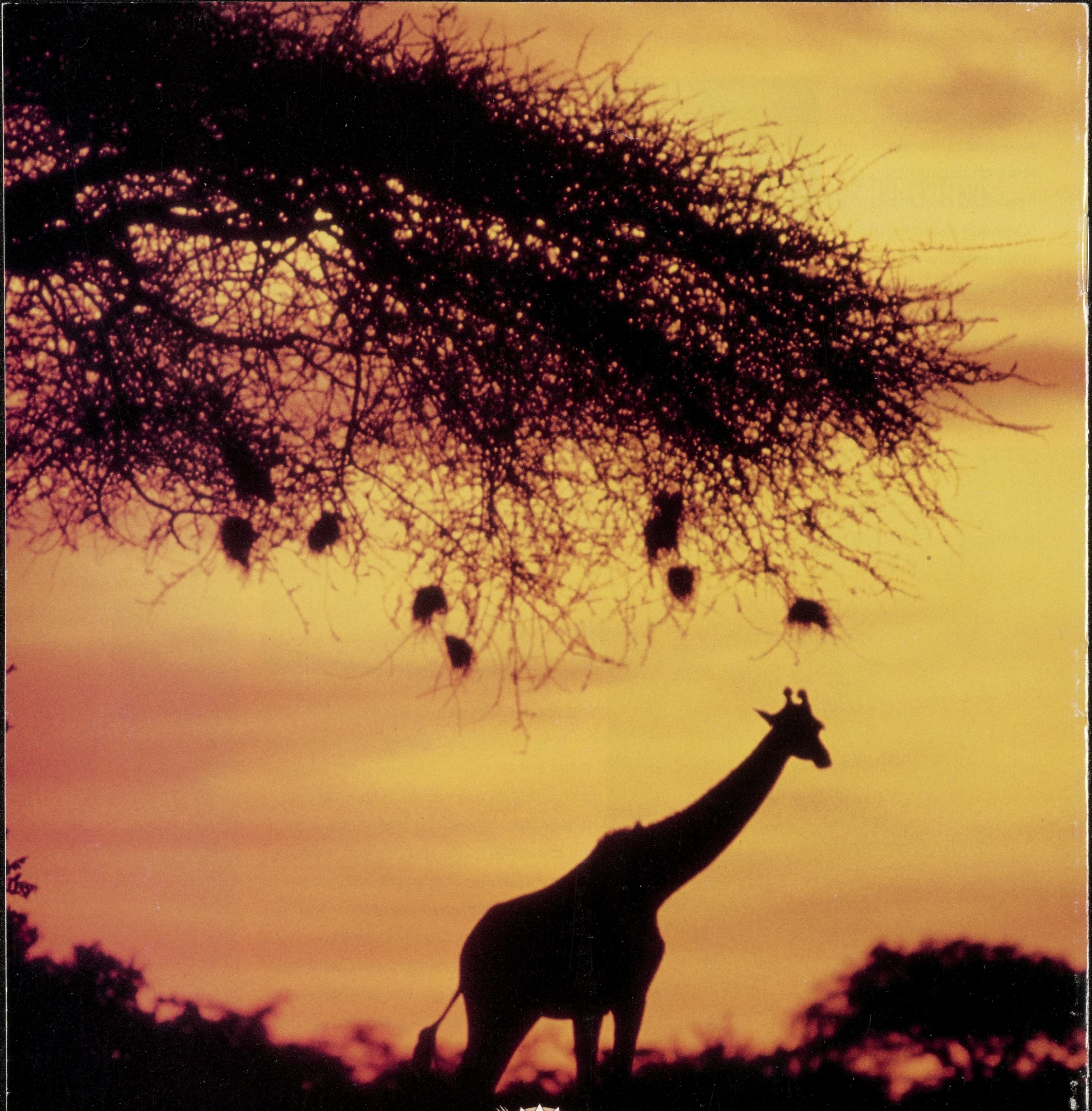
charity begins at home, he decided to lead a pledge drive for the research department headed by his father. It was not Rolf's first involvement with that department. He had worked there in 1975 as a University of California, Davis, college student, and he had been part of the team that established a genetic method for determining the gender of birds.

Rolf called his program Kicks for Critters, and he asked fans to pledge a certain amount for each field goal he kicked. The program caught on immediately with San Diegans, who loved their Chargers almost as much as they loved their Zoo. Established late in the 1980 season, the first year's campaign raised $76,000 for the research department. But more important than the money raised, the campaign made the public aware of the existence of CRES for the first time. "Kicks for Critters made it known nationally," Dr. Benirschke recalled. "The Chargers were doing very well, and that helped."

Kicks for Critters ended with Rolf's football career, but the awareness remained. "Many people who are now regular donors to CRES, if you look back in their file, you find that their first gift was a pledge to Kicks for Critters," said Charles Bieler, former Zoo director and now head of development for the Zoological Society.

Several existing public fund-raising drives are direct spinoffs from Kicks for Critters. One is Cans for Critters, a school-based program for collecting aluminum cans for recycling. Participating schools receive educational material from CRES, and the youngsters definitely get the message. Another ongoing campaign is Celebration for the Critters, a fall fund-raising party now held on Zoo grounds. It started in 1981 as a Kicks for Critters pledge party, and since 1982, it has been a popular fund-raiser in its own right.

All these campaigns, along with funds from large and small donations and product licensing, are dedicated to building up the CRES endowment fund. The endowment is expected to supply the bulk of the program's budget when it is fully funded. It has been a long effort, and it is far from over. The Zoological Society now hopes to expand its system of endowed chairs, modeled after similar programs in universities where an endowment is set up specifically to support one senior researcher. Endowed positions at CRES include the Kleberg Chair in genetics and the Benirschke Chair for the director, as well as the J. Dallas Clark Postdoctoral Fellowship and the Jewell and James Bonner Summer College Student Fellowship Endowment Fund.



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C·R·E·S

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